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(54) Title: QUINAZOLINE DERIVATIVE (57) Abstract The invention concerns the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, processes for its preparation, pharmaceutical compositions containing the compound and the use of the receptor tyrosine kinase inhibitory properties of the compound in the treatment of proliferative disease such as cancer.		

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QUINAZOLINE DERIVATIVE

The invention relates to a quinazoline derivative, or a pharmaceutically-acceptable salt thereof, which possesses anti-proliferative activity such as anti-cancer activity and is accordingly useful in methods of treatment of the human or animal body. The invention also relates to processes for the manufacture of said quinazoline derivative, to pharmaceutical compositions containing the compound and to its use in the manufacture of medicaments of use in the production of an anti-proliferative effect in a warm-blooded animal such as man.

Many of the current treatment regimes for cell proliferation diseases such as psoriasis and cancer utilise compounds which inhibit DNA synthesis. Such compounds are toxic to cells generally but their toxic effect on rapidly dividing cells such as tumour cells can be beneficial. Alternative approaches to anti-proliferative agents which act by mechanisms other than the inhibition of DNA synthesis have the potential to display enhanced selectivity of action.

In recent years it has been discovered that a cell may become cancerous by virtue of the transformation of a portion of its DNA into an oncogene i.e. a gene which, on activation, leads to the formation of malignant tumour cells (Bradshaw, Mutagenesis, 1986, 1, 91). Several such oncogenes give rise to the production of peptides which are receptors for growth factors. The growth factor receptor complex subsequently leads to an increase in cell proliferation. It is known, for example, that several oncogenes encode tyrosine kinase enzymes and that certain growth factor receptors are also tyrosine kinase enzymes (Yarden et al., Ann. Rev. Biochem., 1988, 57, 443; Larsen et al. Ann. Reports in Med. Chem. 1989, Chpt. 13).

Receptor tyrosine kinases are important in the transmission of biochemical signals which initiate cell replication. They are large enzymes which span the cell membrane and possess an extracellular binding domain for growth factors such as epidermal growth factor (EGF) and an intracellular portion which functions as a kinase to phosphorylate tyrosine amino acids in proteins and hence to influence cell proliferation. Various classes of receptor tyrosine kinases are known (Wilks, Advances in Cancer Research, 1993, 60, 43-73) based on families of growth factors which bind to different receptor tyrosine kinases. The classification includes Class I receptor tyrosine kinases comprising the EGF family of

receptor tyrosine kinases such as the EGF, TGF α , NEU, erbB, Xmrk, HER and let23 receptors, Class II receptor tyrosine kinases comprising the insulin family of receptor tyrosine kinases such as the insulin, IGF1 and insulin-related receptor (IRR) receptors and Class III receptor tyrosine kinases comprising the platelet-derived growth factor (PDGF) family of receptor tyrosine kinases such as the PDGF α , PDGF β and colony-stimulating factor 1 (CDF1) receptors. It is known that Class I kinases such as the EGF family of receptor tyrosine kinases are frequently present in common human cancers such as breast cancer (Sainsbury *et al.*, Brit. J. Cancer, 1988, 58, 458; Guerin *et al.*, Oncogene Res., 1988, 3, 21 and Klijn *et al.*, Breast Cancer Res. Treat., 1994, 29, 73), non-small cell lung cancers (NSCLCs) including adenocarcinomas (Cerny *et al.*, Brit. J. Cancer, 1986, 54, 265; Reubi *et al.*, Int. J. Cancer, 1990, 45, 269; and Rusch *et al.*, Cancer Research, 1993, 53, 2379) and squamous cell cancer of the lung (Hendler *et al.*, Cancer Cells, 1989, 7, 347), bladder cancer (Neal *et al.*, Lancet, 1985, 366), oesophageal cancer (Mukaida *et al.*, Cancer, 1991, 68, 142), gastrointestinal cancer such as colon, rectal or stomach cancer (Bolen *et al.*, Oncogene Res., 1987, 1, 149), cancer of the prostate (Visakorpi *et al.*, Histochem. J., 1992, 24, 481), leukaemia (Konaka *et al.*, Cell, 1984, 37, 1035) and ovarian, bronchial or pancreatic cancer (European Patent Specification No. 0400586). As further human tumour tissues are tested for the EGF family of receptor tyrosine kinases it is expected that their widespread prevalence will be established in further cancers such as thyroid and uterine cancer. It is also known that EGF type tyrosine kinase activity is rarely detected in normal cells whereas it is more frequently detectable in malignant cells (Hunter, Cell, 1987, 50, 823). It has been shown more recently (W J Gullick, Brit. Med. Bull., 1991, 47, 87) that EGF receptors which possess tyrosine kinase activity are overexpressed in many human cancers such as brain, lung squamous cell, bladder, gastric, breast, head and neck, oesophageal, gynaecological and thyroid tumours.

Accordingly it has been recognised that an inhibitor of receptor tyrosine kinases should be of value as a selective inhibitor of the growth of mammalian cancer cells (Yaish *et al.* Science, 1988, 242, 933). Support for this view is provided by the demonstration that erbstatin, an EGF receptor tyrosine kinase inhibitor, specifically attenuates the growth in athymic nude mice of a transplanted human mammary carcinoma which expresses EGF receptor tyrosine kinase but is without effect on the growth of another carcinoma which does not express EGF receptor tyrosine kinase (Toi *et al.*, Eur. J. Cancer Clin. Oncol., 1990, 26,

722.) Various derivatives of styrene are also stated to possess tyrosine kinase inhibitory properties (European Patent Application Nos. 0211363, 0304493 and 0322738) and to be of use as anti-tumour agents. The *in vivo* inhibitory effect of two such styrene derivatives which are EGF receptor tyrosine kinase inhibitors has been demonstrated against the growth of human squamous cell carcinoma inoculated into nude mice (Yoneda *et al.*, Cancer Research, 1991, 51, 4430). Various known tyrosine kinase inhibitors are disclosed in a more recent review by T R Burke Jr. (Drugs of the Future, 1992, 17, 119).

It is known from European Patent Applications Nos. 0520722, 0566226 and 0635498 that certain quinazoline derivatives which bear an anilino substituent at the 4-position possess receptor tyrosine kinase inhibitory activity. It is further known from European Patent Application No. 0602851 that certain quinazoline derivatives which bear a heteroaryl amino substituent at the 4-position also possess receptor tyrosine kinase inhibitory activity.

It is further known from International Patent Application WO 92/20642 that certain aryl and heteroaryl compounds inhibit EGF and/or PDGF receptor tyrosine kinase. There is the disclosure of certain quinazoline derivatives therein but no mention is made of 4-anilinoquinazoline derivatives.

The *in vitro* anti-proliferative effect of a 4-anilinoquinazoline derivative has been disclosed by Fry *et al.*, Science, 1994, 265, 1093. It was stated that the compound 4-(3'-bromoanilino)-6,7-dimethoxyquinazoline was a highly potent inhibitor of EGF receptor tyrosine kinase.

The *in vivo* inhibitory effect of a 4,5-dianilinophthalimide derivative which is an inhibitor of the EGF family of receptor tyrosine kinases has been demonstrated against the growth in BALB/c nude mice of a human epidermoid carcinoma A-431 or of a human ovarian carcinoma SKOV-3 (Buchdunger *et al.*, Proc. Nat. Acad. Sci., 1994, 91, 2334). Accordingly it has been indicated that Class I receptor tyrosine kinase inhibitors will prove to be useful in the treatment of a variety of human cancers.

It is also expected that inhibitors of EGF type receptor tyrosine kinases will be useful in the treatment of other diseases of excessive cellular proliferation such as psoriasis.

There is no disclosure in these documents of quinazoline derivatives which bear at the 4-position an anilino substituent and which also bear an alkoxyalkylamino substituent at the 6-position and an alkoxy substituent at the 7-position. We have now found that such a

compound possesses potent *in vivo* anti-proliferative properties which are believed to arise from its Class I receptor tyrosine kinase inhibitory activity.

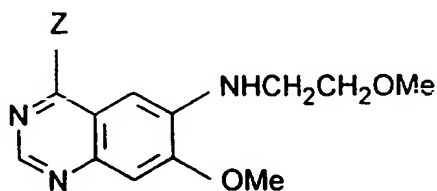
According to the present invention there is provided the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanylino)quinazoline; or a pharmaceutically-acceptable salt thereof.

It is also to be understood that the quinazoline derivative of the invention can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which possess anti-proliferative activity.

A suitable pharmaceutically-acceptable salt of the quinazoline derivative of the invention is, for example, a mono- or di-acid-addition salt of the quinazoline derivative of the invention which is sufficiently basic, for example, an acid-addition salt with, for example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric, maleic, tartaric, fumaric, methanesulphonic or 4-toluenesulphonic acid.

The quinazoline derivative of the invention, or a pharmaceutically-acceptable salt thereof, may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Suitable processes include, for example, those illustrated in European Patent Applications Nos. 0520722, 0566226, 0602851, 0635498 and 635507. Such processes, when used to prepare the quinazoline derivative of the invention, or a pharmaceutically-acceptable salt thereof, are provided as a further feature of the invention and are illustrated by the following representative Example. Necessary starting materials may be obtained by standard procedures of organic chemistry. The preparation of such starting materials is described within the accompanying non-limiting Example. Alternatively necessary starting materials are obtainable by analogous procedures to those illustrated which are within the ordinary skill of an organic chemist.

(a) The reaction, conveniently in the presence of a suitable base, of a quinazoline of the formula I



I

wherein Z is a displaceable group, with 3'-methylaniline.

A suitable displaceable group Z is, for example, a halogeno, alkoxy, aryloxy or sulphonyloxy group, for example a chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-4-sulphonyloxy group.

A suitable base is, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine, N-methylmorpholine or diazabicyclo[5.4.0]undec-7-ene, or, for example, an alkali or alkaline earth metal carbonate or hydroxide, for example sodium carbonate, potassium carbonate, calcium carbonate, sodium hydroxide or potassium hydroxide. Alternatively a suitable base is, for example, an alkali metal or alkaline earth metal amide, for example sodium amide or sodium bis(trimethylsilyl)amide.

The reaction is preferably carried out in the presence of a suitable inert solvent or diluent, for example an alkanol or ester such as methanol, ethanol, isopropanol or ethyl acetate, a halogenated solvent such as methylene chloride, chloroform or carbon tetrachloride, an ether such as tetrahydrofuran or 1,4-dioxan, an aromatic solvent such as toluene, or a dipolar aprotic solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidin-2-one or dimethylsulphoxide. The reaction is conveniently carried out at a temperature in the range, for example, 10 to 150°C, preferably in the range 20 to 80°C.

The quinazoline derivative of the invention may be obtained from this process in the form of the free base or alternatively it may be obtained in the form of a salt with the acid of the formula H-Z wherein Z has the meaning defined hereinbefore. When it is desired to obtain the free base from the salt, the salt may be treated with a suitable base as defined hereinbefore using a conventional procedure.

(b) The alkylation, conveniently in the presence of a suitable base as defined hereinbefore, of 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline with an alkylating agent of the formula ZCH_2CH_2OMe wherein Z is a displaceable group as defined hereinbefore.

The reaction is preferably carried out in a suitable inert solvent or diluent as defined hereinbefore and at a temperature in the range, for example, 0 to 150°C, conveniently at or near ambient temperature.

(c) The reductive amination of 2-methoxyacetaldehyde with 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline.

The reduction may be carried out by any of the many procedures known for such a transformation. For example a hydride reducing agent may be used, for example an alkali metal borohydride or cyanoborohydride such as sodium borohydride or sodium cyanoborohydride, or an alkali metal aluminium hydride such as lithium aluminium hydride. The reduction may be carried out in the presence of a suitable inert solvent or diluent, for example a (1-4C)alcohol such as methanol or ethanol when an alkali metal borohydride or cyanoborohydride is employed, or an inert ether such as diethyl ether or tetrahydrofuran when an alkali metal aluminium hydride is employed.

The reaction is conveniently carried out at a temperature in the range, for example, -10 to 100°C conveniently at or near ambient temperature.

(d) The methylation, conveniently in the presence of a suitable base as defined hereinbefore, of the compound

7-hydroxy-6-(2-hydroxyethylamino)-4-(3'-methylanilino)quinazoline,

7-hydroxy-6-(2-methoxyethylamino)-4-(3'-methylanilino)quinazoline or

6-(2-hydroxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline

with a methylating agent of the formula Me-Z wherein Z is a displaceable group as defined hereinbefore.

The reaction is preferably carried out in a suitable inert solvent or diluent as defined hereinbefore and at a temperature in the range, for example, 0 to 150°C, conveniently at or near 50°C.

When a pharmaceutically-acceptable salt of the quinazoline derivative of the invention is required, for example a mono- or di-acid-addition salt of the quinazoline derivative of the invention, it may be obtained, for example, by reaction of said compound with, for example, a suitable acid using a conventional procedure.

As stated hereinbefore the quinazoline derivative defined in the present invention possesses anti-proliferative activity which is believed to arise from the Class I receptor tyrosine kinase inhibitory activity of the compound. These properties may be assessed, for example, using one or more of the procedures set out below:-

(a) An in vitro assay which determines the ability of a test compound to inhibit the enzyme EGF receptor tyrosine kinase. Receptor tyrosine kinase was obtained in partially purified form from A-431 cells (derived from human vulval carcinoma) by the procedures described below which are related to those described by Carpenter *et al.*, J. Biol. Chem., 1979,

254, 4884, Cohen et al., *J. Biol. Chem.*, 1982, 257, 1523 and by Braun et al., *J. Biol. Chem.*, 1984, 259, 2051.

A-431 cells were grown to confluence using Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS). The obtained cells were homogenised in a hypotonic borate/EDTA buffer at pH 10.1. The homogenate was centrifuged at 400 g for 10 minutes at 0-4°C. The supernatant was centrifuged at 25,000 g for 30 minutes at 0-4°C. The pelleted material was suspended in 30 mM Hepes buffer at pH 7.4 containing 5% glycerol, 4 mM benzamidine and 1% Triton X-100, stirred for 1 hour at 0-4°C, and recentrifuged at 100,000 g for 1 hour at 0-4°C. The supernatant, containing solubilised receptor tyrosine kinase, was stored in liquid nitrogen.

For test purposes 40 µl of the enzyme solution so obtained was added to a mixture of 400 µl of a mixture of 150 mM Hepes buffer at pH 7.4, 500 µM sodium orthovanadate, 0.1% Triton X-100, 10% glycerol, 200 µl water, 80 µl of 25 mM DTT and 80 µl of a mixture of 12.5 mM manganese chloride, 125 mM magnesium chloride and distilled water. There was thus obtained the test enzyme solution.

Each test compound was dissolved in dimethylsulphoxide (DMSO) to give a 50 mM solution which was diluted with 40 mM Hepes buffer containing 0.1% Triton X-100, 10% glycerol and 10% DMSO to give a 500 µM solution. Equal volumes of this solution and a solution of epidermal growth factor (EGF; 20 µg/ml) were mixed.

[γ -³²P]ATP (3000 Ci/mM, 250 µCi) was diluted to a volume of 2 ml by the addition of a solution of ATP (100 µM) in distilled water. An equal volume of a 4 mg/ml solution of the peptide Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly in a mixture of 40 mM Hepes buffer at pH 7.4, 0.1% Triton X-100 and 10% glycerol was added.

The test compound/EGF mixture solution (5 µl) was added to the test enzyme solution (10 µl) and the mixture was incubated at 0-4°C for 30 minutes. The ATP/peptide mixture (10 µl) was added and the mixture was incubated at 25°C for 10 minutes. The phosphorylation reaction was terminated by the addition of 5% trichloroacetic acid (40 µl) and bovine serum albumin (BSA; 1 mg/ml, 5 µl). The mixture was allowed to stand at 4°C for 30 minutes and then centrifuged. An aliquot (40 µl) of the supernatant was placed onto a strip of Whatman p 81 phosphocellulose paper. The strip was washed in 75 mM phosphoric acid (4 x 10 ml) and blotted dry. Radioactivity present in the filter paper was measured using a liquid scintillation counter (Sequence A). The reaction sequence was repeated in the

absence of the EGF (Sequence B) and again in the absence of the test compound (Sequence C).

Receptor tyrosine kinase inhibition was calculated as follows:-

$$\% \text{ Inhibition} = \frac{100 - (A-B)}{C - B} \times 100$$

The extent of inhibition was then determined at a range of concentrations of test compound to give an IC_{50} value.

(b) An *in vitro* assay which determines the ability of a test compound to inhibit the EGF-stimulated growth of the human naso-pharyngeal cancer cell line KB.

KB cells were seeded into wells at a density of 1×10^4 - 1.5×10^4 cells per well and grown for 24 hours in DMEM supplemented with 5% FCS (charcoal-stripped). Cell growth was determined after incubation for 3 days by the extent of metabolism of MTT tetrazolium dye to furnish a bluish colour. Cell growth was then determined in the presence of EGF (10 ng/ml) or in the presence of EGF (10 ng/ml) and a test compound at a range of concentrations. An IC_{50} value could then be calculated.

(c) An *in vivo* assay in a group of athymic nude mice (strain ONU:Alpk) which determines the ability of a test compound (usually administered orally as a ball-milled suspension in 0.5% polysorbate) to inhibit the growth of xenografts of the human vulval epidermoid carcinoma cell line A-431.

A-431 cells were maintained in culture in DMEM supplemented with 5% FCS and 2mM glutamine. Freshly cultured cells were harvested by trypsinization and injected subcutaneously (10 million cells/0.1 ml/mouse) into both flanks of a number of donor nude mice. When sufficient tumour material was available (after approximately 9 to 14 days), fragments of tumour tissue were transplanted in the flanks of recipient nude mice (test day 0). Generally, on the seventh day after transplantation (test day 7) groups of 7 to 10 mice with similar-sized tumours were selected and dosing of the test compound was commenced. Once daily dosing of test compound was continued for a total of 13 days (test days 7 to 19 inclusive). In some studies the dosing of the test compound was continued beyond test day 19, for example to test day 26. In each case, on the following test day the animals were killed

and the final tumour volume was calculated from measurements of the length and width of the tumours. Results were calculated as a percentage inhibition of tumour volume relative to untreated controls.

The compound of the invention possesses activity at approximately the following concentrations or doses in tests (a) to (c):-

Test (a):- IC_{50} 0.01 μ M;

Test (b):- IC_{50} 0.2 μ M;

Test (c):- 50% inhibition of tumour volume from a daily dose of 50 mg/kg.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, in association with a pharmaceutically-acceptable diluent or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository.

In general the above compositions may be prepared in a conventional manner using conventional excipients.

The quinazoline derivative will normally be administered to a warm-blooded animal at a unit dose within the range 5-10000 mg per square meter body area of the animal, i.e. approximately 0.1-200 mg/kg, and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250 mg of active ingredient. Preferably a daily dose in the range of 1-100 mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

According to a further aspect of the present invention there is provided the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, for use in a method of treatment of the human or animal body by therapy.

We have now found that the compound of the present invention possesses anti-proliferative properties such as anti-cancer properties which are believed to arise from its

Class I receptor tyrosine kinase inhibitory activity. Accordingly the compound of the present invention is expected to be useful in the treatment of diseases or medical conditions mediated alone or in part by Class I receptor tyrosine kinases, i.e. the compound may be used to produce a Class I receptor tyrosine kinase inhibitory effect in a warm-blooded animal in need of such treatment. Thus the compound of the present invention provides a method for treating the proliferation of malignant cells characterised by inhibition of Class I receptor tyrosine kinases, i.e. the compound may be used to produce an anti-proliferative effect mediated alone or in part by the inhibition of Class I receptor tyrosine kinase. Accordingly the compound of the present invention is expected to be useful in the treatment of psoriasis and/or cancer by providing an anti-proliferative effect, particularly in the treatment of Class I receptor tyrosine kinase sensitive cancers such as cancers of the breast, lung, colon, rectum, stomach, prostate, bladder, pancreas and ovary.

Thus according to this aspect of the invention there is provided the use of the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, in the manufacture of a medicament for use in the production of an anti-proliferative effect in a warm-blooded animal such as man.

According to a further feature of this aspect of the invention there is provided a method for producing an anti-proliferative effect in a warm-blooded animal, such as man, in need of such treatment which comprises administering to said animal an effective amount of the quinazoline derivative as defined immediately above.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular proliferative disease will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. A unit dose in the range, for example, 1-200 mg/kg, preferably 1-100 mg/kg is envisaged.

The anti-proliferative treatment defined hereinbefore may be applied as a sole therapy or may involve, in addition to the quinazoline derivative of the invention, one or more other anti-tumour substances, for example those selected from, for example, mitotic inhibitors, for example vinblastine, vindesine and vinorelbine; tubulin disassembly inhibitors such as taxol; alkylating agents, for example cis-platin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, tegafur, methotrexate, cytosine arabinoside and hydroxyurea, or, for example, one of the preferred antimetabolites disclosed in European Patent Application No. 239362 such as N-{5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-

ylmethyl)-N-methylamino]-2-thenoyl}-L-glutamic acid; intercalating antibiotics, for example adriamycin, mitomycin and bleomycin; enzymes, for example asparaginase; topoisomerase inhibitors, for example etoposide and camptothecin; biological response modifiers, for example interferon; and anti-hormones, for example antioestrogens such as tamoxifen or, for example antiandrogens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl)propionanilide. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment. According to this aspect of the invention there is provided a pharmaceutical product comprising the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, and an additional anti-tumour substance as defined hereinbefore for the conjoint treatment of cancer.

As stated above the quinazoline derivative defined in the present invention is an effective anti-cancer agent, which property is believed to arise from its Class I receptor tyrosine kinase inhibitory properties. Such a quinazoline derivative of the invention is expected to possess a wide range of anti-cancer properties as Class I receptor tyrosine kinases have been implicated in many common human cancers such as leukaemia and breast, lung, colon, rectal, stomach, prostate, bladder, pancreas and ovarian cancer. Thus it is expected that the quinazoline derivative of the invention will possess anti-cancer activity against these cancers. It is in addition expected that the quinazoline derivative of the present invention will possess activity against a range of leukaemias, lymphoid malignancies and solid tumours such as carcinomas and sarcomas in tissues such as the liver, kidney, prostate and pancreas.

It is further expected that the quinazoline derivative of the invention will possess activity against other diseases involving excessive cellular proliferation such as psoriasis, benign prostatic hypertrophy (BPH) and atherosclerosis.

It is also to be expected that the quinazoline derivative of the invention will be useful in the treatment of additional disorders of cellular growth in which aberrant cell signalling by way of receptor tyrosine kinase enzymes, including as yet unidentified receptor tyrosine kinase enzymes, are involved. Such disorders include, for example, inflammation, angiogenesis, vascular restenosis, immunological disorders, pancreatitis, kidney disease and blastocyte maturation and implantation.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids such as drying agents by filtration, unless otherwise stated magnesium sulphate was used as a drying agent for organic solutions;

(ii) operations were carried out at ambient temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) melting points were determined using a Mettler SP62 automatic melting point apparatus, an oil-bath apparatus or a Koffler hot plate apparatus.

(vi) the structure of the compound of the invention was confirmed by nuclear (generally proton) magnetic resonance (NMR) and mass spectral techniques; proton magnetic resonance chemical shift values were measured on the delta scale and peak multiplicities are shown as follows: s, singlet; d, doublet; t, triplet; m, multiplet, unless otherwise stated end-products of the invention were dissolved in CD₃SOCD₃ for the determination of NMR values;

(vii) intermediates were not generally fully characterised and purity was assessed by thin layer chromatography (TLC), infra-red (IR) or NMR analysis;

(viii) the following abbreviations have been used:-

DMF N,N-dimethylformamide;

DMSO dimethylsulphoxide.

Example 1

Concentrated hydrochloric acid (0.75 ml) was added to a stirred mixture of 2-methoxyacetaldehyde dimethyl acetal (9.15 ml) and water (40 ml) and the resultant solution was heated to 40°C for 4 hours. The solution was cooled to ambient temperature and basified to pH8 by the addition of a saturated aqueous sodium bicarbonate solution. The mixture so obtained was added to a stirred suspension of 6-amino-7-methoxy-4-(3'-methylanilino)-quinazoline (4 g) in a mixture of ethanol (500 ml) and glacial acetic acid (0.81 ml). The mixture was stirred at ambient temperature for 30 minutes. Sodium cyanoborohydride (1.78 g) was added and the mixture was stirred at ambient temperature for 16 hours. The acidity of the mixture was increased to pH3.4 by the addition of glacial acetic acid (1 ml) and a second portion (0.5 g) of sodium cyanoborohydride was added. The mixture was stirred at ambient temperature for 2 hours. The acidity of the mixture was again increased to pH3.4 by the addition of glacial acetic acid (2 ml) and a third portion (1 g) of sodium cyanoborohydride was added. The mixture was stirred at ambient temperature for 3 hours. The mixture was basified to pH8 by the addition of a saturated aqueous sodium bicarbonate solution and the mixture was evaporated. The residue was purified by column chromatography using increasingly polar mixtures of methylene chloride and methanol as eluent. The material so obtained was dissolved in methylene chloride and the solution was washed with water and with a dilute aqueous ammonium hydroxide solution. The organic phase was dried (MgSO_4) and evaporated. There was thus obtained 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline (3.81 g, 76%);

NMR Spectrum: 2.35 (s, 3H), 2.45 (m, 2H), 3.28 (s, 3H), 3.52 (t, 2H), 3.98 (s, 3H), 5.32 (t, 1H), 6.9 (m, 1H), 7.08 (s, 1H), 7.25 (t, 1H), 7.30 (s, 1H), 7.39 (m, 1H), 7.65 (m, 1H), 8.35 (s, 1H), 9.5 (broad s, 1H);

Elemental Analysis: Found C, 64.7; H, 6.7; N, 15.9;

$\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_2 \cdot 0.8\text{H}_2\text{O}$ requires C, 64.7; H, 6.4; N, 15.9%.

The 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline used as a starting material was obtained as follows:-

A mixture of 4-chloroanthranilic acid (17.2 g) and formamide (10 ml) was stirred and heated to 130°C for 45 minutes and to 175°C for 75 minutes. The mixture was allowed to cool to approximately 100°C and 2-(2-ethoxyethoxy)ethanol (50 ml) was added. The solution

so formed was poured into a mixture (250 ml) of ice and water. The precipitate was isolated, washed with water and dried. There was thus obtained 7-chloro-3,4-dihydroquinazolin-4-one (15.3 g, 85%).

A portion (6 g) of the material so obtained was added portionwise to a stirred mixture of concentrated sulphuric acid (12 ml) and fuming nitric acid (12 ml) which had been cooled to 0°C. The mixture was stirred at ambient temperature for 30 minutes and then heated to 110°C for 30 minutes. The mixture was cooled to ambient temperature and poured onto a mixture of ice and water. The precipitate was isolated, washed with water and dried. There was thus obtained 7-chloro-6-nitro-3,4-dihydroquinazolin-4-one (6.89 g).

A mixture of a portion (4 g) of the material so obtained, thionyl chloride (30 ml), phosphoryl chloride (5 ml) and DMF (10 drops) was stirred and heated to reflux for 4 hours. The mixture was evaporated, toluene (20 ml) was added and the mixture was re-evaporated. There was thus obtained 4,7-dichloro-6-nitroquinazoline as a solid which was used without further purification.

A mixture of the solid so obtained, 3'-methylaniline (1.89 g) and isopropanol (25 ml) was stirred and heated to reflux for 2 hours. The mixture was filtered whilst still hot. The solid so isolated was washed with isopropanol and with diethyl ether and dried. There was thus obtained 7-chloro-4-(3'-methylanilino)-6-nitroquinazoline hydrochloride (3.74 g), m.p. 271-274°C.

After appropriate repetition of the previous steps, sodium methoxide (15.4 g) was added portionwise to a stirred mixture of 7-chloro-4-(3'-methylanilino)-6-nitroquinazoline (20 g) and DMSO (200 ml) and at a rate such that the temperature of the reaction mixture did not exceed 25°C. The mixture was stirred at ambient temperature for 3 hours. The mixture was poured into water (800 ml) and the precipitate was isolated, washed with water and dried. There was thus obtained 7-methoxy-4-(3'-methylanilino)-6-nitroquinazoline (16.2 g, 90%).

A mixture of the material so obtained, 10% palladium-on-carbon (1.5 g) and ethanol (1 litre) was stirred under an atmosphere of hydrogen for 16 hours. The mixture was filtered and the filtrate was evaporated. The residue was triturated under a 100:3 mixture of methylene chloride and methanol. The resultant solid was isolated and dried. There was thus obtained 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline (9.9 g, 68%);
NMR Spectrum: 2.33 (s, 3H), 3.97 (s, 3H), 5.23 (broad s, 2H), 6.67 (d, 1H), 7.08 (s, 1H), 7.23 (m, 1H), 7.43 (s, 1H), 7.64 (d, 2H), 8.32 (s, 1H), 9.13 (broad s, 1H).

Example 2

The following illustrate representative pharmaceutical dosage forms containing the compound of the invention, or a pharmaceutically-acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)	<u>Tablet I</u>	<u>mg/tablet</u>
	Compound X.....	100
	Lactose Ph.Eur.....	182.75
	Croscarmellose sodium.....	12.0
	Maize starch paste (5% w/v paste)	2.25
	Magnesium stearate	3.0
(b)	<u>Tablet II</u>	<u>mg/tablet</u>
	Compound X.....	50
	Lactose Ph.Eur.....	223.75
	Croscarmellose sodium.....	6.0
	Maize starch.....	15.0
	Polyvinylpyrrolidone	2.25
	Magnesium stearate	3.0
(c)	<u>Tablet III</u>	<u>mg/tablet</u>
	Compound X.....	1.0
	Lactose Ph.Eur.....	93.25
	Croscarmellose sodium.....	4.0
	Maize starch paste (5% w/v paste)	0.75
	Magnesium stearate	1.0

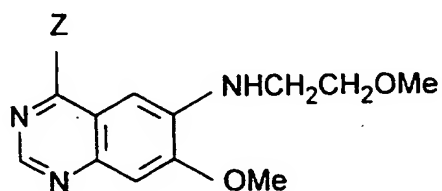
(d)	<u>Capsule</u>	<u>mg/capsule</u>
	Compound X.....	10
	Lactose Ph. Eur.....	488.5
	Magnesium stearate	1.5
(e)	<u>Injection I</u>	<u>(50 mg/ml)</u>
	Compound X.....	5.0% w/v
	1M Sodium hydroxide solution	15.0% w/v
	0.1M Hydrochloric acid (to adjust pH to 7.6)	
	Polyethylene glycol 400	4.5% w/v
	Water for injection to 100%	
(f)	<u>Injection II</u>	<u>(10 mg/ml)</u>
	Compound X.....	1.0% w/v
	Sodium phosphate BP.....	3.6% w/v
	0.1M Sodium hydroxide solution	15.0% v/v
	Water for injection to 100%	
(g)	<u>Injection III</u>	<u>(1mg/ml buffered to pH6)</u>
	Compound X.....	0.1% w/v
	Sodium phosphate BP.....	2.26% w/v
	Citric acid.....	0.38% w/v
	Polyethylene glycol 400	3.5% w/v
	Water for injection to 100%	

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

CLAIMS

1. The quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)-quinazoline;
or a pharmaceutically-acceptable salt thereof.
2. A process for the preparation of the quinazoline derivative as claimed in claim 1 which comprises:-
 - (a) the reaction of a quinazoline of the formula I



wherein Z is a displaceable group, with 3'-methylaniline;

- (b) the alkylation of 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline with an alkylating agent of the formula ZCH_2CH_2OMe wherein Z is a displaceable group;
- (c) the reductive amination of 2-methoxyacetaldehyde with 6-amino-7-methoxy-4-(3'-methylanilino)quinazoline; or
- (d) the methylation of the compound 7-hydroxy-6-(2-hydroxyethylamino)-4-(3'-methylanilino)quinazoline. 7-hydroxy-6-(2-methoxyethylamino)-4-(3'-methylanilino)quinazoline or 6-(2-hydroxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline with a methylating agent of the formula $Me-Z$ wherein Z is a displaceable group;

and when a pharmaceutically-acceptable salt of the quinazoline derivative is required it may be obtained by reaction of said compound with a suitable acid using a conventional procedure.

3. A pharmaceutical composition which comprises the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, in association with a pharmaceutically-acceptable diluent or carrier.

4. The quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, for use in a method of treatment of the human or animal body by therapy.
5. The use of the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, in the manufacture of a medicament for use in the production of an anti-proliferative effect in a warm-blooded animal.
6. A method for producing an anti-proliferative effect in a warm-blooded animal in need of such treatment which comprises administering to said animal an effective amount of the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)-quinazoline, or a pharmaceutically-acceptable salt thereof.
7. A pharmaceutical product comprising the quinazoline derivative 6-(2-methoxyethylamino)-7-methoxy-4-(3'-methylanilino)quinazoline, or a pharmaceutically-acceptable salt thereof, and an additional anti-tumour substance for the conjoint treatment of cancer.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/00962

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D239/94 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 566 226 (ZENECA LIMITED) 20 October 1993 cited in the application see whole document, especially page 3, line 38 and page 12, lines 1-9 ---	1-7
Y	EP,A,0 635 498 (ZENECA LIMITED) 25 January 1995 cited in the application see whole document, especially definition of R1, page 5, line 35 ---	1-7
A	DRUGS OF THE FUTURE, vol. 17, no. 2, 1992, pages 119-131, XP002007460 T.R.BURKE JNR.: "Protein tyrosine kinase inhibitors" cited in the application ---	1-7

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Date of the actual completion of the international search

4 July 1996

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

I. International Application No
PCT/GB 96/00962

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	<p>J.MED.CHEM, vol. 39, 5 February 1996, pages 267-276, XP002007461 A.J.BRIDGES ET AL: "Tyrosine kinase inhibitor" see whole document, especially Table 1, p 269, entry 46</p> <p>-----</p>	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 96/00962

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-566226	20-10-93	AT-T- 130000	15-11-95
		AU-B- 661533	27-07-95
		AU-B- 3101093	22-07-93
		CA-A- 2086968	21-07-93
		DE-D- 69300754	14-12-95
		DE-T- 69300754	28-03-96
		ES-T- 2078798	16-12-95
		NZ-A- 245662	26-09-95
		SK-A- 1693	09-09-93
		US-A- 5457105	10-10-95
		ZA-A- 9300015	20-07-93
		JP-A- 6073025	15-03-94
EP-A-635498	25-01-95	AU-B- 7191694	20-02-95
		WO-A- 9503283	02-02-95
		US-A- 5475001	12-12-95
		ZA-A- 9405156	19-01-95

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